EFFECT OF SALINITY ON GERMINATION PARAMETERS OF NATURAL POPULATIONS OF *Lolium* (POACEAE)

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Abstract. In the framework of the evaluation, valorization and conservation of plant genetic resources, six natural populations belonging to two species of forage grasses, perennial ryegrass (*Lolium perenne* L.) and annual ryegrass (*Lolium rigidum* Gaud.) collected in different agro-ecological regions of Algeria, were used to assess the effects of four NaCl and Na_2SO_4 concentrations (0, 50, 100, 150 mM) on germination parameters: germination percentage (GP), germination rate index (GRI), mean germination time (MGT), salt tolerance index (STI), seedling vigor index (SVI), shoot and root length. Seeds were germinated in an incubator at 20°C for 12 days in a completely randomized design with five replicates. The results revealed significant effects of NaCl and Na_2SO_4, populations and their interactions on germination parameters. Salinity reduced GP, STI, VI, GRI, shoot and root length, but increased MGT, in contrast to control experiment (0 mM). An important decrease was globally observed in the two species in all parameters with the lowest values at 100 and 150 mM except MGT. Compared to NaCl, the decrease was more important under Na_2SO_4. Population LP1 of *L. perenne* and the three populations of *L. rigidum* in the control and in the two salts showed the highest means of GP, GRI and SVI. Populations LR3 of *L. rigidum* (arid area) and LP3 of *L. perenne* (sub humid area) were the most sensitive at 150 mM. The shorter MGT was obtained in population LP1 of *L. perenne* which seems to be more salt-tolerant toward the two salts than the annual *L. rigidum.*

Key words: *Lolium perenne* L.; *Lolium rigidum* Gaud.; germination; salinity stress; natural populations.

INTRODUCTION

Soil salinization and poor water quality are a global problem for agricultural production [3]. The increase of salt contents in aquatic and terrestrial ecosystems is one of the major consequences of ongoing climate change [1]. This problem which affects nearly 6% (1 billion hectares) of the land area worldwide [8] and more than 20% of irrigated soils area in Algeria [20], is particularly important in arid and semi-arid regions [42]. Cl^-, Na^+ and SO_4^{2-} are among predominant soil ions in semi-arid and arid areas which represent the largest part of Algeria. The increase of soil osmotic potential is exacerbated by high temperature and evapotranspiration during summers [30, 64].

Salinity affects plant growth at all stages of development [27] and reduces persistence, yield and biomass accumulation in many crops, including forage crops [61]. Salt inhibits seed germination either by creating an osmotic potential external to the seed preventing water uptake or by high concentrations of sodium and chloride ions in the soil [38]. The influence of salts on germination is complicated by the interactions of osmotic and ion effects [69].

Seed germination is the first crucial step in the plant life cycle [49] and germinating seeds are the most sensitive to salinity stress [37], its success is decisive throughout the rest of the vegetative stage [66]. Genetic variations in salt tolerance exist, and the degree of salt tolerance varies with plant species and varieties within a species [29]. Individual and within populations genetic variability, is an essential factor of long term adaptability [50] and is considered as one of the fundamental requirements for plant breeding [67]. To restore productivity of saline ecosystem, selection and introduction of multipurpose salt tolerant plants with desirable characteristics is necessary [7].

The genus *Lolium* L. (Poaceae) is native to Europe, temperate Asia and North Africa [14]. The annual species *Lolium rigidum* Gaud. is an important annual, self-reseeding forage species widespread in the natural pastures of semi-arid Mediterranean environments [26]. Cultivar Wimmera known for its high degree of salt tolerance [74], traditionally utilized as a pasture species has become the most problematic and difficult to control weed and even has been found to occur in summer across grain production regions in Australia [76]. *Lolium perenne* L. (perennial ryegrass) forms the basis of grassland production [55] and is the premier cultivated pasture of temperate regions worldwide [13]. It is a high-yielding with excellent forage quality under frequent cutting regime in many humid climate regions [68]. Highly palatable fodders resources [51, 53], these two species are part of the spontaneous grasses that often constitute the essential component of pastures, meadows and fallows in Algeria. They are characterized by significant genetic variability [58] and a strong tendency to structure populations according to eco-geographical conditions [6].

The survival of plants during germination and emergence is considered as the most important sign of tolerance to salt [40]. The response of seeds to salinity is an indicator of plant tolerance [72] for selection of genotypes adapted and plant breeding.

Algeria is confronted with the challenges of soil erosion, which affects the quantity and quality of soil [31] and the unavailability of fodder for extended periods of the year [56]. Furthermore, climatic constraints have a negative impact on livestock yields. Exploring the diversity of spontaneous plant genetic resources is a crucial step in selecting adapted plant species and rehabilitating land degraded by salinity. Restoration and utilization of saline rangelands in Algeria are urgent ecological and economic solutions [59]. By using plant genetic resources (PGRs), crop varieties are being developed with better yield and quality traits along with resistance to biotic and abiotic stresses, such as diseases, insect pests, flooding, salinity and drought [70].

The existing studies indicate considerable variability in response to salinity stress and need to be investigated in germplasm collections to identify potential lines for salinity stress tolerance [46].

The objective of the present work was to evaluate the response to salinity induced with different concentrations of NaCl and Na_2SO_4 on germination parameters of six natural populations belonging to two species of forage grasses, perennial ryegrass (*Lolium perenne* L.) and annual ryegrass *(Lolium rigidum* Gaud.) originating from various agro-ecological regions of Algeria.

MATERIALS AND METHODS

Plant material and experimental procedures

Six natural populations of the genus *Lolium* (3 of *Lolium perenne* and 3 of *Lolium rigidum*) (Fig 1) collected from different agro-ecological sites in Algeria (Table 1) were studied at the National Institute of Agronomic Research of Algeria (INRAA). Mature and healthy caryopses, were sterlized in a 1.5% hypochloric solution for 3 minutes, rinced in distilled water and then gentely dried with wiping paper. Salt solutions (0, 50, 100 and 150 mM) were prepared with 2 neutral salts (NaCl and Na_2SO_4). These concentrations correspond to saline soils levels of arid and semi-arid areas of Algeria [30, 65]. For each salt solution and replication, 10 seeds were placed in a Petri dish (60 mm Ø) (Fig 1) with two layers of filter paper previousely moistured wih 6 ml of solution or distilled water for control (0 mM).

In total, 50 caryopses were used for every concentration. The germination experiment was conducted in a complete randomization with five replicates. All Petri dishes were kept in a programmed incubator set at 20°C and a relative humidity of 65% in the dark for 12 days. Black plastic bags were used to provide complete (24 h) dark condition. Caryopses were considered germinated when the radicle emerged 2 mm [54]. Germinated caryopses were counted daily until no further germination was observed (12 days.). Shoots and roots of 10 seedlings were weighed and measured with a tape ruler.

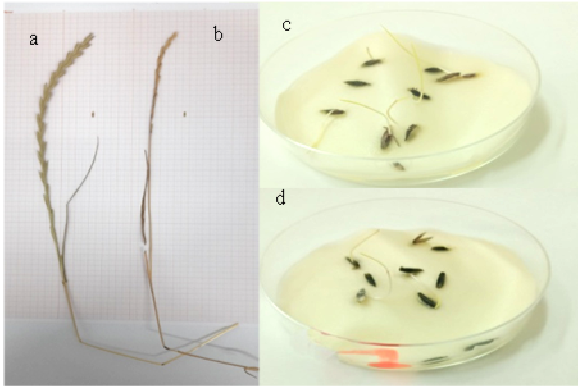

Figure 1. Image showing the spike and seed of the plant material used: *Lolium rigidum* (a), *Lolium perenne* (b) and germinated *Lolium perenne* seeds under 50 mM (c) and 150 mM (d) NaCl concentrations.

Germination parameters and methods of calculation

For each replication, the germination percentage (GP) (%) is the number of germinated seeds of the total incubated seeds and was calculated as:

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total of number seeds}} \times 100$$

Germination rate index (GRI) was estimated by using a modified Timson (1965) [77] index of germination velocity after Khan and Ungar (1984) [41] following the equation:

$$GRI = \Sigma\, G/t$$

where *G* is the percentage of seeds germinated at 2-day intervals, and *t* is the total germination period (Easton and Kleindorfer, 2009) [21].

The mean germination time (MGT) is the necessery mean time for seeds to germinate and was calculated as:

$$MGT = \Sigma\,(Ti \times Ni)/\Sigma Ni$$

where *Ni* is the newly germinated seeds at time *Ti* following Alvarado *et al.* (1987) formula [5].

The salt tolerance index (STI) was measured to capture the differences in salt tolerance among genotypes and can be estimated for various morphological parameters [8]. This parameter is the ratio of shoot-root total fresh weight of the salt concentration and total fresh weight of the control treatment. The total fresh weight (*TFW*) was mesured on ten seedlings. The STI was calculated

Table 1. Geographical origins and characteristics of the studied populations of *Lolium perenne* and *Lolium rigidum*

Species/populations	Code	Province	Alt. (m)	P (mm)	Tm (°C)	Bioclimate
Lolium perenne						
Population 1	LP1	Constantine	700	501.17	15.3	Semi-arid
Population 2	LP2	Skikda	338	679.94	18.26	Sub humid
Population 3	LP3	Algiers	200	615	18.2	Sub humid
Lolium rigidum						
Population 1	LR1	Boumerdès	10	672	17.6	Sub humid
Population 2	LR2	Sétif	986	583	13.5	Semi-arid
Population 3	LR3	Djelfa	1020	273	17.9	Arid

Note: Alt. (m): Altitude, P(mm): annual average rainfall, Tm(°C): annual average temperature.

by the following formula according to Kusvuran *et al.* (2014) [48]:

$$STI = (TFWtr)/(TFWcr) \times 100$$

where STI is the salt tolerance index, *TFWtr* is the mean of the total fresh weight under salt concentration condition (at 50, 100, 150 mM) and *TFWcr* refers to the mean of the total fresh weight under control treatment (at 0 mM).

Ten seedling were used to estimate seedling vigor index (SVI) according to Belwal *et al.* (2015) [10] as:

$$SVI = GP \times SL$$

where *SL* is the seedling (shoot and root) length in cm and *GP* represents the germination percentage.

Data analysis

Data obtained from percentage germination counts were first subjected to an angular arcsine transformation and then used for statistical test. The effect of populations, salinity and their interaction on germination percentages at day 12 (arcsine transformed data) and all studied traits were subjected to factorial anlysis of variance (ANOVA) using STATISTICA software (version 8.0) followed by Duncan Post-hoc test to compare the means (±sd) with 5% probability level.

RESULTS

The analysis of variance (ANOVA) revealed significant effects of NaCl and Na_2SO_4, populations and their interactions on germination parameters. Salinity reduced germination percentage (GP), vigor index (VI), germination rate index (GRI) and shoot and root length but increased mean germination time (MGT). For the STI, populations showed variation at different salt concentrations.

Germination percentage (GP)

Salt stress affected considerably the germination percentage (GP) showing significant variability among *Lolium* populations (Table 2). Germination capacity of the two *Lolium* species studied in conditions without salt stress was similar, and reached 100%, while population LP3 of *L. perenne* showed the lowest average (80%). In salt treatment, the GP decreased significantly depending on the salt type (NaCl, Na_2SO_4), concentration and population. Under 150 mM NaCl and Na_2SO_4, GP was reduced in all populations ranging from 0 to 58%. Compared to NaCl, the decrease was more important under Na_2SO_4 which induced the lowest germination rate at all concentrations.

Within *Lolium perenne,* under salt treatment, the highest germination percentage reached up to 94% and was found in population LP1 at 50 mM NaCl and did not exceed 64% at 50 mM NaCl in population LP3 which is characterized by the lowest values at all concentrations. Overall results demonstrated higher GP in annual ryegrass populations under the two treatments and population LP1 of perennial ryegrass under NaCl. This was particularly evident at higher concentrations (150 mM) of Na_2SO_4 with GP reaching 32, 58 and 26% compared to *Lolium perenne*. Population LR3 collected from arid area showed the lowest GP among *Lolium rigidum* populations.

Germination Rate Index

The results of germination rate index (GRI) demonstrated significant differences between treatments, interactions population x treatment and among populations of *Lolium* species. However, differences were noticeably important within perennial compared to annual ryegrass populations. As the concentrations doses of both treatments increased, the GRI decreased with lower means under Na_2SO_4 (Table 3). The highest averages were obtained at 0 mM to 100 mM in perennial ryegrass collected from Constantine region (semi-arid), followed by annual populations and then by populations LP2 and LP3.

Mean Germination Time

The mean germination time (MGT) showed a large variation between all populations and the salinity levels. The MGT was shorter in the control treatment and increased significantly with increasing concentrations of NaCl and Na_2SO_4 (Table 4) for all populations except LP3 of the perennial species whose germination was completely inhibited at 150 mM Na_2SO_4.

Under NaCl-stress conditions, the MGT extreme averages were obtained in *L. perenne* (LP1, LP3) and were more prolonged at 150 mM (LP1, LP2) and at 100 mM in LP3 under Na_2SO_4. Within annual ryegrass, the effects of populations and the interaction population x treatment was less important. MGT was significantly influenced by salinity and was longer under Na_2SO_4 than NaCl specially at 150 mM (populations LR1 and LR3). Conversely, population LR2 from semi-arid reacted with longer MGT under NaCl compared to Na_2SO_4 at 150 mM.

Table 2. Effects of different levels of salinity on germination percentage (GP, %) of *Lolium* species

Treatment	(C)	*Lolium perenne*			*Lolium rigidum*		
		LP1	LP2	LP3	LR1	LR2	LR3
NaCl	0 mM	100(0.00)^{a}	94(5.47)^{a}	80(0.00)^{c}	100(0.00)^{a}	100(0.00)^{a}	100(0.00)^{a}
	50 mM	94(5.47)^{a}	78(8.36)^{c}	64(5.47)^{def}	90(7.07)^{bc}	88(8.36)^{bc}	84(11.40)^{bcd}
	100 mM	92(8.36)^{ab}	72(8.36)^{cde}	44(15.16)^{fg}	76(15.16)^{bcde}	90(7.07)^{b}	60(7.07)^{fg}
	150 mM	78(13.03)^{cd}	40(12.24)^{gf}	28(4.47)^{g}	66(8.94)^{efg}	40(21.21)^{h}	50(14.14)^{gh}
Na_2SO_4	0 mM	100(0.00)^{a}	94(5.47)^{a}	80(00.00)^{c}	100(0.00)^{a}	100(0.00)^{a}	100(0.00)^{a}
	50 mM	80(14.14)^{bc}	80(14.14)^{c}	52(8.36)^{f}	70(7.07)^{def}	88(13.03)^{b}	76(11.40)^{cdef}
	100 mM	58(8.36)^{ef}	52(13.03)^{ef}	12(13.03)^{h}	62(17.88)^{efg}	80(14.14)^{bcd}	60(7.07)^{fg}
	150 mM	10(17.32)^{hi}	26(15.16)^{g}	0.00(0.00)^{i}	32(8.36)^{h}	58(8.36)^{g}	26(11.40)^{h}

Note: Data are expressed as % are mean (SD) of five replicates per population (genotype). Value followed by different letters are different by the Duncan's test (P<0.05), (C): Concentration.

Table 3. Effects of different levels of salinity on germination rate index (GRI) of *Lolium* species

Treatment	(C)	*Lolium perenne*			*Lolium rigidum*		
		LP1	**LP2**	**LP3**	**LR1**	**LR2**	**LR3**
NaCl	0 mM	17.58(1.79)^{a}	13.91(2.72)^{b}	9.86(1.26)^{de}	16.51(1.33)^{a}	14.81(0.94)^{ab}	15.46(0.60)^{ab}
	50 mM	16.18(0.60)^{a}	11.30(0.96)^{cd}	6.88(1.38)^{fg}	13.61(1.20)^{bc}	10.92(1.78)^{def}	11.31(2.04)^{cde}
	100 mM	12.44(1.42)^{bc}	9.79(1.48)^{de}	4.93(2.35)^{g}	10.86(1.26)^{def}	11.78(1.10)^{cd}	9.00(2.78)^{efghi}
	150 mM	7.90(1.26)^{ef}	5.16(2.18)^{g}	2.29(0.36)^{h}	8.54(1.48)^{fghi}	5.04(2.55)^{jkl}	7.69(2.78)^{ghi}
Na_2SO_4	0 mM	17.58(1.79)^{a}	13.91(2.72)^{b}	9.86(1.26)^{de}	16.51(1.33)^{a}	14.81(2.70)^{ab}	15.46(0.68)^{ab}
	50 mM	13.47(2.17)^{b}	12.16(2.97)^{bc}	5.04(0.88)^{g}	11.00(1.38)^{def}	11.47(1.55)^{cde}	9.41(3.02)^{defgh}
	100 mM	5.70(1.66)^{g}	4.78(1.13)^{g}	0.7(0.81)^{hi}	9.85(2.58)^{defg}	11.08(2.76)^{def}	7.28(1.79)^{hij}
	150 mM	0.10(0.01)^{i}	1.47(0.68)^{hi}	0.00(0.00)^{i}	4.49(1.40)^{kl}	6.76(1.32)^{ijk}	2.96(1.12)^{l}

Note: Data are expressed as mean (SD) of five replicates per population (genotype). Value followed by different letters are different by the Duncan's test (P<0.05), (C): Concentration.

Table 4. Effects of different levels of salinity on mean germination time (MGT, days) of *Lolium* species

Treatment	(C)	*Lolium perenne*			*Lolium rigidum*		
		LP1	**LP2**	**LP3**	**LR1**	**LR2**	**LR3**
NaCl	0 mM	7.22(0.08)^{i}	7.58(0.13)^{ghi}	8.04(0.20)^{efg}	7.46(0.20)^{f}	7.72(0.05)^{ef}	7.65(0.07)^{ef}
	50 mM	7.39(0.08)^{hi}	7.72(0.21)^{ghi}	8.32(0.22)^{def}	7.62(0.18)^{ef}	8.09(0.24)^{abcd}	7.80(0.05)^{de}
	100 mM	7.93(0.25)^{hi}	7.89(0.41)^{fgh}	8.36(0.41)^{def}	7.92(0.17)^{abcde}	7.92(0.19)^{abcde}	7.81(0.04)^{cde}
	150 mM	8.51(0.12)^{cde}	8.0(30.39)^{efg}	9.00(0.00)^{bc}	7.93(0.24)^{abcde}	8.24(0.51)^{a}	7.87(0.06)^{bcde}
Na_2SO_4	0 mM	7.22(0.08)^{i}	7.58(0.13)^{ghi}	8.04(0.20)^{efg}	7.46(0.20)^{f}	7.72(0.05)^{ef}	7.65(0.07)^{ef}
	50 mM	7.41(0.07)^{hi}	7.63(0.29)^{ghi}	8.48(0.33)^{cde}	7.65(0.05)^{ef}	7.86(0.36)^{bcde}	8.07(0.06)^{abcd}
	100 mM	8.50(0.23)^{cde}	8.69(0.18)^{cd}	10.22(0.73)^{a}	7.70(0.28)^{ef}	7.90(0.21)^{abcde}	8.09(0.05)^{abcd}
	150 mM	10.4(1.14)^{a}	9.46(0.30)^{b}	0.00(0.00)^{j}	8.20(0.26)^{ab}	8.14(0.13)^{abcd}	8.16(0.54)^{abc}

Note: Data are expressed as days are mean (SD) of five replicates per population (genotype). Values followed by different letters are different by the Duncan's test (P<0.05).test (P<0.05), (C): Concentration.

Salt tolerance index

For both species the population effect was not significant. The interaction population x treatment was not significant within annual ryegrass but significant within perennial ryegrass. The values of the STI index were highest in population LP3 at 50 and 100 mM under the two salt treatments with population LP1 at 50 mM Na_2SO_4 and the lowest in population LP2. When exposed to the 150 mM stress regimes, population LP2 appears to be the most tolerant with the highest values. The highest STI were recorded in populations LR3 and LR1 (at 50 and 100 mM NaCl) and LR3 and LR2 at 50 mM Na_2SO_4 followed by LR1 and LR2 at 100 mM Na_2SO_4.

Seedling Vigor Index

Salt doses, populations and their interactions impacted importantly seedling vigor index (SVI) within the two species of *Lolium*. With the increasing of salinity, a decrease of SVI was particularly observed under Na_2SO_4 (Table 6). Under salt treatments, the highest values of SVI were observed in all populations of annual *Lolium*. Perennial *Lolium* populations were characterized by the lowest SVI which varied from 596.3 (LP1) to 110.7 (LP3) under NaCl and from 553.9 (LP2) to 0 (LP1, LP3) under Na_2SO_4.

Table 5. Effects of different levels of salinity on salt tolerance index (STI, %) of *Lolium* species

Treatment	(C)	*Lolium perenne*			*Lolium rigidum*		
		LP1	**LP2**	**LP3**	**LR1**	**LR2**	**LR3**
NaCl	50 mM	101.48(32.88)^{ab}	82.30(18.01)^{bcd}	111.95(18.16)^{a}	88.72(9.92)^{ab}	80.02(15.10)^{ab}	96.48(24.33)^{a}
	100 mM	65.86(12.48)^{def}	81.89(16.22)^{cd}	90.79(23.52)^{bc}	78.86(18.31)^{ab}	75.31(26.29)^{abc}	77.41(16.91)^{abc}
	150 mM	64.70(14.05)^{defg}	69.05(10.05)^{de}	68.98(19.84)^{de}	73.74(17.44)^{abc}	73.21(28.67)^{abc}	66.01(22.53)^{bc}
Na_2SO_4	50 mM	79.50(11.2)^{cd}	71.87(6.53)^{cd}	78.5(11.50)^{cd}	66.37(11.11)^{bc}	70.17(16.92)^{bc}	70.62(19.41)^{bc}
	100 mM	48.53(7.15)^{efg}	47.25(7.95)^{fg}	60.22(5.99)^{defg}	53.50(6.77)^{cd}	53.69(13.93)^{cd}	41.27(12.53)^{d}
	150 mM	0.00(0.00)^{h}	44.50(9.76)^{g}	0.00(0.00)^{h}	0.00(0.00)^{e}	0.00(0.00)^{e}	0.00(0.00)^{e}

Note: Data are expressed as % are mean (SD) of ten replicates per population (genotype). Values followed by different letters are different by the Duncan's test (P<0.05), (C): Concentrations.

Table 6. Effects of different levels of salinity on salt vigor index (SVI) of *Lolium* species

Treatment	(C)	*Lolium perenne*			*Lolium rigidum*		
		LP1	**LP2**	**LP3**	**LR1**	**LR2**	**LR3**
NaCl	0 mM	715.20(77.00)^{a}	722.28(130.88)^{a}	544.80(22.87)^{b}	913.00(14.40)^{b}	1266.00(34.16)^{a}	1393.00(39.46)^{a}
	50 mM	596.30(104.51)^{b}	549.40(84.14)^{b}	396.70(59.89)^{c}	853.40(156.30)^{bc}	947.50(127.01)^{b}	1219.40(358.58)^{a}
	100 mM	503.00(52.02)^{b}	539.20(76.13)^{b}	202.50(101.43)^{d}	660.00(325.30)^{cd}	868.70(102.59)^{b}	640.90(153.47)^{d}
	150 mM	378.60(84.01)^{c}	175.20(52.70)^{de}	110.70(28.80)^{def}	512.00(118.15)^{de}	372.40(213.26)^{ef}	497.60(119.48)^{de}
Na_2SO_4	0 mM	715.20(77.00)^{a}	722.28(130.88)^{a}	544.80(22.87)^{b}	913.00(14.40)^{b}	1266.00(34.16)^{a}	1393.00(39.46)^{a}
	50 mM	545.00(141.48)^{b}	553.90(142.09)^{b}	337.90(28.17)^{c}	620.60(33.38)^{d}	942.40(167.52)^{b}	941.06(184.12)^{b}
	100 mM	402.10(97.43)^{c}	128.40(35.06)^{de}	33.90(37.73)^{efg}	283.50(42.71)^{f}	515.90(188.25)^{de}	385.10(114.73)^{ef}
	150 mM	0.00(0.00)^{g}	18.35(15.44)^{fg}	0.00(0.00)^{g}	0.00(0.00)^{g}	0.00(0.00)^{g}	0.00(0.00)^{g}

Note: Data are expressed as mean (SD) of five replicates per population (genotype). Values followed by different letters are different by the Duncan's test (P<0.05), (C): Concentrations.

Shoot and root length

Increasing NaCl and Na_2SO_4 concentrations resulted in a decrease in shoot and root elongation except at 50 mM (Table 7). While the longest shoot length was found in all populations of annual ryegrass, particularly LR3 collected in arid area, the lowest mean (0.55 cm) was determined at 100 mM Na_2SO_4 in LP3 originating from sub humid site. High salt concentration completely inhibited the germination and no shoot were recorded in both species

Population LR3 of annual ryegrass also showed longer roots (6.24 cm) at 50 mM NaCl. The lowest reductions of root length were detected in the population LP2 (0.58 cm) at 150 mM Na_2SO_4 which considerably inhibited root development of all other populations. In general, greater reduction in shoot and root length was observed in populations studied due to Na_2SO_4 compared to NaCl.

DISCUSSION

Effect of salinnity on seed germination

Adaptation to saline-alkaline environment during seed germination is crucial for plant establishment. A low water potential caused by osmotic and the ionic effects are determining factors inhibiting seed germination under salinity stress [19]. In the basis on our study, the *Lolium* populations varied in their response to salinity in germination and seedling characteristics. Both annual and perennial ryegrass had higher germination percentage in distilled water than in the two saline treatments. However, the lowest values of GP and the mortality of the seeds were only observed at very high concentrations (100 and 150 mM) suggesting that low salinity did not endanger *L. perenne* and *L. rigidum* germination. Several previous works reported similar results. Xu *et al.* (2020) [81] found that more than half of seeds of *L. perenne* could finally germinate at salinity up to 12.8‰. Dai *et al.* (2009) [17] and Kusvuran *et al.* (2014) [48] stated that *L. perenne* was relatively the most salt-tolerant among the tested several cool-season turfgrass species at high salinity levels. Under these same salinity conditions, a strong inhibition of the germination of *L. perenne* was also found by Borawska-Jarmułowicz *et al.* (2017) and Borowski *et al.* (2008) [11, 12]. Similar findings reported a decrease in germination of *Lolium rigidum* and *Lolium perenne* seeds with increasing NaCl salinity [15, 34]. Khalid (2018) [39] confirmed the sensitivity of both species to water salinity, with low germination at 100 mM of NaCl and poor to absent germination at higher concentrations. Our results are in agreement with those reported by Khan and Ungar (1984) [41] who demonstrated that the germination percentage decreased progressively with increasing salinity in *Atriplex triangularis* and those reported by Kokten *et al.* (2010) [44], who observed a significant difference in the salt tolerance of lentil genotypes.

In our study, sodium sulfate appeared to have a greater effect on germination than did sodium chloride. Undersander (1986) [79] has also shown that the germination of several Poaceae species (crested wheatgrass, tall fescue, bluegrama, perennial ryegrass, Russian wildrye) was reduced by sodium sulfate treatments but not by equivalent levels of sodium chloride. Nedjimi *et al*. (2020) [60] found that the inhibition by soluble salts on seed germination on *Marrubium vulgare* L. was in the following order: $Na_2SO_4 > MgCl_2 > CaCl_2 > NaCl$. In contrary, Vicente *et al*. (2009) [80] and Zhumabekova *et al*. (2020) [85] attested that NaCl prevents germination respectively of three plant species growing in salt marsh soils of semi-arid Mediterranean environments and *Haloxylon ammodendron* more than Na_2SO_4. The difference between salts was probably due either to the higher concentration of sodium ions or to the slightly lower

Table 7. Effects of different levels of salinity on length shoot-root of *Lolium* species

Treatment	(C)	**Shoot** (cm)					
		Lolium perenne			***Lolium rigidum***		
		LP1	**LP2**	**LP3**	**LR1**	**LR 2**	**LR 3**
NaCl	0 mM	3.03(0.83)^{de}	5.95(1.17)^{a}	3.84(0.60)^{bc}	6.34(0.44)^{c}	6.80(1.69)^{bc}	7.60(0.99)^{ab}
	50 mM	3.16(0.45)^{d}	4.16(1.25)^{b}	3.36(1.08)^{cd}	5.35(0.60)^{de}	6.32(0.89)^{c}	8.15(1.80)^{a}
	100 mM	2.47(0.29)^{ef}	3.89(0.45)^{bc}	1.77(0.71)^{ghi}	4.72(0.94)^{efg}	5.34(0.86)^{de}	6.35(1.18)^{c}
	150 mM	1.92(0.76)^{gh}	2.07(0.31)^{fg}	1.59(0.73)^{ghi}	4.19(2.63)^{fg}	4.86(0.68)^{efg}	4.96(0.74)^{ef}
Na_2SO_4	0 mM	3.03(0.83)^{de}	5.95(1.17)^{a}	3.84(0.60)^{bc}	6.34(0.44)^{c}	6.80(1.69)^{bc}	7.60(0.99)^{ab}
	50 mM	3.38(0.32)^{cd}	3.58(0.61)^{bcd}	3.44(0.80)^{cd}	6.01(0.85)^{cd}	5.96(0.99)^{cd}	6.92(0.88)^{bc}
	100 mM	1.14(0.42)^{i}	1.35(0.49)^{hi}	0.55(0.58)^{j}	3.07(0.50)^{g}	4.33(1.37)^{fg}	3.97(0.95)^{g}
	150 mM	0.00(0.00)^{j}	0.06(0.18)^{j}	0.00(0.00)^{j}	0.00(0.00)^{i}	0.00(0.00)^{i}	0.00(0.00)^{i}
		Root (cm)					
		Lolium perenne			***Lolium rigidum***		
		LP1	**LP2**	**LP3**	**LR1**	**LR 2**	**LR 3**
NaCl	0 mM	3.42(0.54)^{abc}	3.15(0.49)^{bcde}	2.69(0.49)^{def}	2.86(0.68)^{fg}	4.47(1.37)^{cde}	5.83(1.14)^{ab}
	50 mM	3.99(0.89)^{a}	2.95(0.89)^{bcdef}	2.85(0.34)^{cdef}	4.08(0.98)^{cde}	4.42(0.89)^{cde}	6.24(1.66)^{a}
	100 mM	3.02(0.55)^{bcdef}	3.61(0.72)^{ab}	2.73(1.13)^{def}	3.65(1.51)^{def}	4.31(0.70)^{cde}	4.29(1.73)^{cde}
	150 mM	2.93(0.76)^{bcdef}	2.50(0.84)^{ef}	2.38(0.59)^{f}	3.50(1.44)^{ef}	4.22(1.27)^{cde}	5.19(1.20)^{bc}
Na_2SO_4	0 mM	3.42(0.54)^{abc}	3.15(0.49)^{bcde}	2.69(0.49)^{def}	2.86(0.68)^{fg}	4.47(1.37)^{cde}	5.83(1.14)^{ab}
	50 mM	3.37(0.55)^{abcd}	3.29(0.65)^{bcd}	3.18(0.78)^{bcde}	2.91(0.42)^{fg}	4.73(0.86)^{cd}	5.79(1.38)^{ab}
	100 mM	1.15(0.66)^{g}	1.22(0.47)^{g}	0.77(1.00)^{g}	1.68(0.52)^{h}	1.97(1.05)^{gh}	2.40(1.20)^{gh}
	150 mM	0.00(0.00)^{i}	0.58(0.10)^{h}	0.00(0.00)^{hi}	0.00(0.00)^{i}	0.00(0.00)^{i}	0.00(0.00)^{i}

Note: Data are expressed as cm are mean (SD) of ten replicates per population (genotype). Values followed by by different letters are different by the Duncan's test ($P<0.05$), (C): Concentrations.

osmotic potential of sodium sulfate treatments but not by equivalent levels of sodium chloride [79].

Germination rate index

Both the MGT and GI can be acceptable parameters to be used in evaluating salt tolerance during germination stage [57]. Significant differences were recorded between treatments, interaction population x treatment and among populations of *Lolium* species with a reduced germination rate index specifically under Na_2SO_4. However, differences were noticeably important within *Lolium perenne* compared to annual *Lolium* populations. This is in agreement with the results of Kohar *et al.* (2022) [45] who concluded that germination percentage and germination rate of maize hybrids were linearly decreased by the decrease in osmotic potential of NaCl solution. Such results have been obtained on *Lolium perenne* L. whose GRI declined with high salinity concentrations [34]. As the concentrations doses of both treatments increased, the GRI decreased with lower values under Na_2SO_4.

A higher GRI expresses a faster germination rate [21] and indicates important percentages and rates of germination [73]. The LP1 population (perennial ryegrass), which has the highest germination percentages under NaCl, also has the lowest reduction in germination index under both saline conditions and seems to perform better.

Mean germination time

Our results revealted that both species were characterized by shorter MGT in control condition, meanwhile a longer MGT was observed with the increasing salt stress. This is in agreement with the results on annual ryegrass cultivars under salt stress prolonged as the salt concentration increased in the germination area [24]. Nizam (2011) [61] and Borawska-Jarmułowicz *et al*. (2017) [11] also stated that MGT of *L. perenne* was significantly increased by salinity. Tonguç *et al.* (2021) [78] on *Carthamus tinctorius* L. (Asteraceae) and Oz (2022) [62] on *Satureja thymbra* (Lamiaceae), pointed out the increase of MGT with increasing salt concentrations. It was also concluded that NaCl increased mean germination time in seeds of *Chloris gayana* Kunth and *Avena sativa* L. [16, 47].

Moreover, we found that MGT was globally significantly longer under Na_2SO_4 than NaCl in both species. Similar results were obtained on *Urochondra setulosa* (Poaceae) [71] and *Phaseolus vulgaris* L. (Fabaceae) [37]. Some studies have also shown that a prolonged MGT under salt stress may be caused by a temporary delay in germination or forced dormancy by osmosis [52] and can be considered as an adaptive strategy for seeds to survive under harsh conditions [28].

Salt tolerance index

The main best advantage of STI index is its ability to discriminate group from other groups [25]. The statistical analysis showed significant differences for NaCl and Na_2SO_4 and the interaction treatment x population in *L. perenne* and salt treatement in *L. rigidum*.

We found that both species studied have a better tolerance to NaCl compared to Na_2SO_4 and are in agreement with those reported by Nedjimi *et al*. (2020) [60] on *Marrubium vulgare* (Lamiaceae). Kokten *et al*. (2010) [44] stated that tolerant genotypes of lentil showed higher salt tolerance indices than sensitive ones. The STI displayed that a perennial ryegrass has a better tolerance toward the two salt treatments particularly population LP3 from sub humid region (at 50 and 100 mM) compared to annual ryegrass. Similar variation was found among varieties of *Brassica napus* (Cruciferae), *Hordeum vulgare* and *Festuca rubra* L.(Poaceae) [8, 22, 48].

Undersander (1986) [79] certified that perennial ryegrass was fairly tolerant to salinity during germination. Tenikecier and Ates (2022) [75] reported that cultivar 'Ringles'of perennial ryegrass with other Poaceae demonstrated good salt tolerance. Significant intra- and inter-specific variation for salt tolerance was indicated by Markar (1987) [54] in the genus *Lolium* during germination.

Seedling vigor index

Seed vigor is a vital index for seed quality that determines particularly the potential for rapid and uniform emergence of plants [33]. In our study, increasing of NaCl and Na_2SO_4 concentrations inversely affected the vigor index in the two species. Ibrahim *et al*. (2019) [33] found that high levels of soil salinity causes reduction in seed vigor in water spinach. Increasing concentrations of NaCl altered critically SVI in *Haloxylon ammodendron* (Chenopodiaceae) populations originating from Kazakhstan and China desert [85]. Low averages of SVI were recorded with high salinity levels for some *Sorghum* forage cultivars [36] and rice (*Orysa sativa*) cultivars [35]. Plants respond differently to salt stress depending on their genetic structure and the severity of the stress [83]. Our SVI results showed various interactions (population x treatment) noticeably with NaCl in *Lolium rigidum* and Na_2SO_4 in *Lolium perenne* which is consistent with reports on *Haloxylon ammodendron* from three different desert areas [85] and wheat genotypes [43]. The SVI varied differently under NaCl than under Na_2SO_4 which gives the greatest reduction in this parameter, in accordance with the findigs of Zhang and Zhao (2011) [84] on *Zea mays* L. cultivars.

Shoot and root length

Root and shoot length is the most significant traits for salinity tolerance because roots have direct contact with soil and absorb water and nutrients from soil [23]. Increasing salinity level decreased root length for both species with large differences among the populations. This result confirmed previous works on several Poaceae (*Oryza sativa* L., *Lolium multiflorum* Lam., and *Lolium perenne* L.) [57, 63, 81]. In general, greater reduction in shoot and root length due to Na_2SO_4 compared to NaCl was observed in population studied which was already found on *Nigella sativa* L. and *Sesamum indicum* L. [2, 32]. Osmotic stress leads to a reduction in root water uptake and the accumulation and toxicity of certain ions causes ionic stress and consequently reduced growth rates and ultimately plant death [35]. Under salt stress, Na^+, Cl^- and SO_4^{2-} can be taken up by the seed and toxic effects of NaCl and Na_2SO_4 can occur [2]. Salt stress affects plant by reducing the water potential of plants and depreciates photosynthesis, reduces root epidermal cell elongation and production, induces turgor loss and ion imbalance in cells, especially lowering concentrations of K^+, $Ca^{2+}$and NO_3^- [4]. This results in an intense competition between K^+, (necessary for protein synthesis and water relations) and Na^+ at the root uptake sites [9]. Ion regulation is an essential factor of the mechanism of salt tolerance in plants. Ability of plant genotypes to maintain higher levels of K^+ and Ca^{2+} and low levels of Na^+ and Cl^- within tissue is one of the key mechanisms contributing to expression of high salt tolerance [18].

We noted less reduction influence of salt treatment at 50 mM compared to the control. Dasgan and Koc (2009) and Yasar *et al.* (2006) [18, 82] stated that some varieties of beans are less affected and grow equally with the control plants and no inhibition effects are caused under saline growth.

In the present study, application of increasing doses of Na_2SO_4 and NaCl caused significant reductions in germination parametrers (GP, GRI, SVI, STI) and shoot and root length, but an elongation of MGT. Regarding the statistical analysis, Na_2SO_4 had more inhibitive action than NaCl on germination parameters. LP3 (*Lolium perenne*) and LR3 (*Lolium rigidum*) from sub humid and arid regions respectively were most sensitive to both salts at high concentrations compared to population LP1 (*Lolium perenne*) originating from semi-arid region.

This significant variability obtained in *Lolium perenne* and *Lolium rigidum* under salt stress involved all the parameters studied, but in particular germination percentage (GP), germination rate index (GRI) and seedling vigor index (SVI). These traits are highly reproducible and may be suitable for evaluating salt stress tolerance in perennial and annual ryegrass species in future breeding programmes. This study opens further perspectives for research on salt tolerance of other local populations to understand the mechanisms of salt stress at all stages of plant development before selecting the best populations to use in different agro-ecological regions.

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